Gingival β_2-microglobulin in juvenile and chronic periodontitis

Stina Syrjänen, Helena Markkanen and Kari Syrjänen
Department of Oral Pathology and Radiology, Department of Periodontology, Faculty of Dentistry, and Department of Pathology, University of Kuopio, Kuopio, Finland

Syrjänen S, Markkanen H, Syrjänen K. Gingival β_2-microglobulin in juvenile and chronic periodontitis. Acta Odontol Scand 1985;43:133–138. Oslo. ISSN 0001–6357.

The β_2-microglobulin (β_2-m) pattern in gingival biopsy specimen from 24 patients with chronic severe periodontitis (P), from 11 patients with juvenile periodontitis (JP), and from 24 periodontally healthy subjects (CO) was studied with an indirect immunoperoxidase method. No reactivity for β_2-m was found in 71% of specimens in the P and CO groups, whereas 82% of the JP specimens showed positive β_2-m staining in the epithelium. The reactivity was detected mostly in the upper layers of the epithelium. In all the three groups the β_2-m reactivity was less frequent in the subepithelial connective tissue than in the epithelium proper, and it seemed to be confined to the inflammatory cells. In the JP group, prominent reactivity for β_2-m was also located in intercellular bridges of the squamous cells. The significance of the results is discussed in terms of the cell differentiation in these diseases, including the function of β_2-m related to the function of the classical HLA antigens (HLA-A, HLA-B, HLA-C). □ *Gingival epithelium; immunostaining*

Stina Syrjänen, Faculty of Dentistry, University of Kuopio, P.O. Box 6, 70211 Kuopio, Finland

Beta$_2$-microglobulin (β_2-m) is a low molecular weight protein (11,700) recently shown to be equivalent to the constant part of the light chain of the HLA type I antigens (that is, the classical HLA antigens: HLA-A, HLA-B, HLA-C). It has 30% homology to the C_H3 region of the IgG chain (1–3). However, β_2-m is coded by chromosome 15, whereas HLA type I antigens are coded by chromosome 6 (4). Accordingly, it has been demonstrated on the surface of all normal human nucleated cells (5). Small amounts of β_2-m are also found in the serum and in saliva (5–7). The exact function of β_2-m is unknown, although the functions seemed to be related to HLA type I functions. It has been suggested that this protein as a constituent of HLA antigens is a part of immune defense system involved in the recognition of cells altered by infection, ageing, or malignant transformation (9). In addition, β_2-m might have a role in intracellular transport of HLA type I antigens and in the expression of the three-dimensional structure of the heavy chain. Elevation of serum β_2-m has been reported in renal tubular disorders, in renal transplant rejection, in autoimmune diseases, and in oral malignancy (10–13). Increased β_2-m concentration of the saliva has been measured in patients with rheumatoid arthritis or Sjögren's syndrome (7, 8). In addition, the positive staining for β_2-m in cutaneous tumors has been shown to be related to tumor differentiation (14–16).

This study was designed to determine the location and distribution of gingival β_2-m in periodontitis and in healthy subjects. The aim was to obtain further information on the immunological mechanisms involved in the development of gingival lesions in chronic and juvenile periodontitis.

Materials and methods

The gingival specimens were obtained from patients evaluated at the Department of Periodontology, University of Kuopio. The subjects were 11 patients with juvenile or postjuvenile periodontitis (JP) and 24 patients with severe periodontitis (P) (17). The diagnosis of JP was determined by clinical and radiological examinations. Patients with severe periodontitis were characterized

by pronounced alveolar bone loss with deep periodontal pocket formations (>6 mm). The series was supplemented with 24 controls (CO) presented with clinically healthy periodontium. The age and sex distribution of the subjects studied is shown in Table 1.

All patients were subjected to gingival biopsy directed at the interdental papilla between the second bicuspid and the first molar in the mandible. The biopsy specimens were fixed in 10% neutral formalin, embedded in paraffin, and prepared for light microscopy in accordance with routine procedures. The 4-μm-thick serial sections were stained with hematoxylin and eosin for grading the inflammatory cell infiltration (graded as absent, slight, moderate, or strong).

The distribution pattern of β_2-microglobulin in the gingival biopsy specimens was demonstrated by an indirect immunoperoxidase (IP-PAP) technique, performed with standard procedures. In brief, the 4-μm-paraffin sections were deparaffinized in xylene, and the endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxidase in methanol for 30 min. To reduce the nonspecific binding of primary antibodies, the sections were incubated with normal goat serum (1:5 dilution in phosphate-buffered saline (PBS)) (Nordic Immunology, Tilburg, The Netherlands) for 30 min at room temperature. Subsequently, the sections were sequentially incubated with rabbit anti-human β_2-m (1:50 dilution in PBS) (Dakopatts a/s, lot A072, Glostrup, Denmark), goat anti-rabbit whole serum (1:40) (Miles Laboratories, Elkhart, Ind., USA), and rabbit peroxidase–anti-peroxidase (PAP) (1:100) (Miles Laboratories). As a substrate for the peroxidase DAB (3 mg/ml 3,3-diaminobenzidine in 0.01% H_2O_2 in PBS) was used, and the sections were incubated for 5 min at room temperature. The antibody Dako titer for β_2-m was 60, indicating the amount of antigen (in μg) which must be added to 1 ml of antiserum to obtain a supernatant containing neither antigen nor antibody. The titer of β_2-m antiserum measured by single radial immunodiffusion (SPI) was 250. No counterstain was used, and the sections were mounted as usual. Parallel negative control sections were processed, the primary antiserum being omitted in one, and DAB incubation only used in the other, to disclose the residual endogenous peroxidase activity. As a positive reaction for β_2-m, a reddish-brown precipitate was observed (Figs. 1 and 2). In the negative controls, no specific reactivity was found.

The staining intensity of β_2-m was classified as negative (0), slightly positive (1), moderately positive (2), or strongly positive (3). Negative denoted no staining at all; slightly positive denoted the sections with a few cells showing staining (generally weak

Table 1. Mean ages and female to male ratios of the patients studied

Series	No. of patients	Mean age, years (± SD)	F/M ratio
JP	11	27.7 ± 11.0	1.25:1
P	24	44.4 ± 7.9	1.4:1
CO	24	32.2 ± 5.6	1.4:1

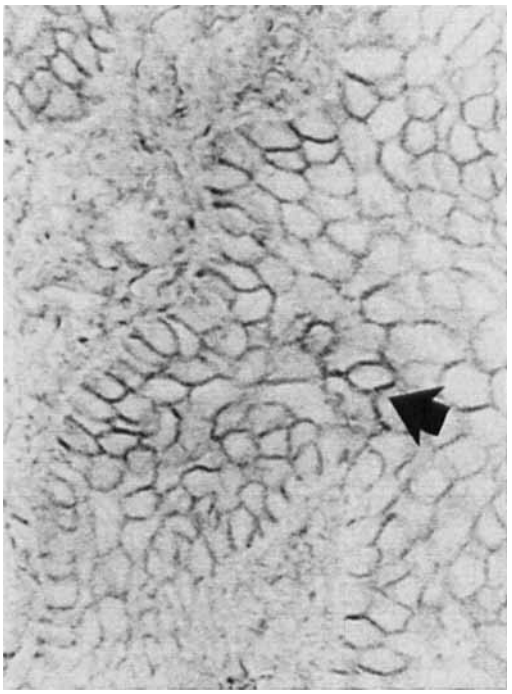

Fig. 1. Gingival epithelium from a JP patient, stained for β_2-m. Intercellular bridges of the squamous cells are clearly seen (arrow), containing the positive reactivity (IP-PAP, original magnification, ×400).

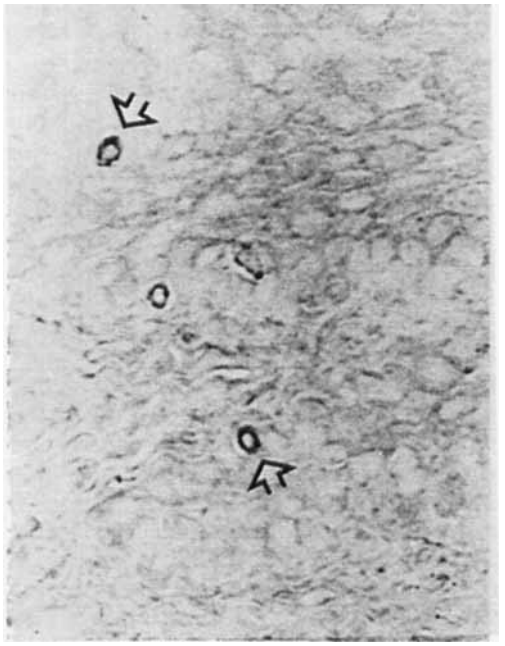

Fig. 2. Some of the lymphatic cells infiltrating the squamous epithelium are clearly visualized by their granular, membrane-bound reactivity for β_2-microglobulin (IP-PAP), original magnification, ×400).

for β_2-m). In moderate staining, 50% of the cells showed staining, generally with a moderate intensity, and the strong staining indicated that most cells were stained, showing an intense brown granular precipitate on their surfaces. All the histological grading procedures were completed blindly. The grading procedure was performed separately for the epithelium and the underlying connective tissue. Furthermore, the staining pattern of epithelial cells was classified as epithelial or intercellular. The latter was graded as slight, moderate, and strong. For statistical analysis the chi-square test was used, and IP and P groups were tested against CO group.

Results

The localization and intensity of β_2-m staining for the three groups studied are presented in Tables 2–4. Seventy-one per cent of the 24 patients with periodontitis and of the controls were negative for β_2-m in oral gingival epithelium, in contrast to only 18% for JP patients, the difference being significant ($p < 0.05$) (Table 2). In all the groups with positive reaction for β_2-m the intensity of the staining was most frequently slight. The positive staining reaction was localized on the cell surfaces and mostly in the upper layers of the oral gingival squamous epithelium (Fig. 1). There was a strong tendency for a positive β_2-m reactivity in the intercellular bridges of oral gingival epithelium in JP group, whereas in the two other groups this staining pattern was not as prominent (Table 3) (Fig. 2) ($p < 0.005$). The staining pattern of β_2-m in pocket epithelium is not reported in this study owing to the lack of the pocket epithelium in some of the specimens.

A positive staining for β_2-m in gingival connective tissue was seen more frequently in the JP series than in the controls or in the patients with chronic periodontitis (Table 4). In addition, the intensity of the staining was also stronger in the JP group than in the two others ($p < 0.05$). The positive staining was generally distributed diffusely on cell surfaces among the inflammatory cells. A slight,

Table 2. β_2-m immunostaining of oral gingival epithelium

Series	No. of cases	Intensity of staining* 0 No.	0 %	1 No.	1 %	2 No.	2 %	3 No.	3 %	Level of significance
JP	11	2	18.2	5	45.5	3	27.3	1	9.1	$p < 0.05$
P	24	17	70.8	4	16.7	3	12.5	0	0.0	NS
CO	24	17	70.8	6	25.0	1	4.2	0	0.0	

* 0 = negative; 1 = slightly positive; 2 = moderately positive; and 3 = strongly positive.

Table 3. Intercellular bridges staining pattern in oral gingival epithelium

Series	No. of cases	Intensity of staining: Slight No.	Slight %	Moderate No.	Moderate %	Strong No.	Strong %	Level of significance
JP	11	0	0.0	2	18.2	9	81.8	$p < 0.005$
P	24	16	66.7	6	25.0	2	8.3	NS
CO	24	13	54.2	8	33.3	3	12.5	

Table 4. β_2-m immunostaining of gingival connective tissue associated with inflammatory cell surfaces

Series	No. of cases	Intensity of staining*: 0 No.	0 %	1 No.	1 %	2 No.	2 %	3 No.	3 %	Level of significance
JP	11	4	36.4	4	36.4	3	27.3	0	0.0	$p < 0.05$
P	24	18	75.0	5	20.8	1	4.2	0	0.0	NS
CO	24	17	70.8	7	29.2	0	0.0	0	0.0	

0 = negative; 1 = slightly positive; 2 = moderately positive; and 3 = strongly positive.

positive correlation was found between the degree of inflammation and β_2-m immunostaining in the connective tissue, but no such correlation could be seen in the epithelial staining (Table 5).

Discussion

The distribution and location of gingival β_2-m in two groups of patients with periodontal diseases and in their healthy counterparts were compared. All the specimens with positive staining (Table 2) showed a tendency to be stained in the upper cell layers of the oral gingival epithelium only. In all three groups positive β_2-m reactivity was found, although the intensity and frequency were most marked in the JP group. The role, if any, played by β_2-m in the pathogenesis of gingival inflammation is not known. Since many researchers have reported that the β_2-m pattern in cells is positively related to the degree of cell differentiation (14, 18–20), it could be assumed that the squamous epithelial cells in the gingiva of JP subjects might represent more mature cell forms than in the other two groups. However, we recently used the immunoperoxidase staining to study the cytokeratin patterns in gingival epithelium of the same series, and we found no disturbed maturation of the epithelial cells (21). Our results were interpreted to suggest that the slight changes in epithelial keratinization detected in the controls and the subjects with periodontal diseases could be due to secondary effects attributed to inflammation (21). However, the possibility of an accel-

Table 5. Correlation between β_2-microglobulin staining and inflammation in gingival connective tissue

Intensity* of β_2-m	The degree of inflammation (no. of cases): Slight (1)	Moderate (2)	Strong (3)
0	4 JP	0 JP	0 JP
	13 P	5 P	0 P
	14 Co	3 Co	0 Co
1	1 JP	3 JP	0 JP
	1 P	4 P	0 P
	5 Co	2 Co	0 Co
2	0 JP	3 JP	0 JP
	0 P	0 P	1 P
	0 Co	0 Co	0 Co

* None of the specimens had a grade 3 stainability.

erated cell turnover could not be excluded (21).

As shown in Fig. 2 and Table 3, β_2-m was positive in the intercellular bridges of the epithelial squamous cells of oral gingiva more frequently in the JP group than in the two others. This might indicate that β_2-m is produced by the epithelial cells themselves, and the detected elevation in β_2-m reactivity in the JP group might reflect an increased production of this protein or a more rapid cell turnover. In addition, the present results might indicate an increased intracellular transport of HLA type I antigens in the JP group, and/or the expression of a three-dimensional structure of the heavy chain could be altered in the oral epithelium of JP patients (1–4, 15, 16). However, further studies are needed to ascertain whether the possible overproduction of β_2-m in JP would also result in excessive shedding of β_2-m in gingival fluid and/or saliva (7, 8). Such a shedding has been one of the most favored hypotheses to explain the elevated serum β_2-m levels detected in connection with a broad spectrum of malignancies and autoimmune diseases as well (7–12, 22, 23). In addition, elevated β_2-m levels have been shown in B-cell lymphoproliferative states, which could also explain the increased intensity found in JP series (22, 24). This is substantiated by our previous results, showing the majority of gingival inflammatory cells in the JP group to be of B-cell linkage (25).

The possibility of HLA antigens are involved in the defense mechanisms of oral gingival epithelium must also be considered. The present results, with increased stainability for β_2-m in the JP group, may provide some evidence for this theory. However, further studies of the controlling mechanisms involved in cell interactions are needed.

The possibility that the variations in β_2-m staining could partly have technical causes is excluded by negative controls. In addition, the IP-PAP method has been shown to be fully applicable in detecting the membrane β_2-m in routinely fixed paraffin-embedded tissues (14). On the basis of our present study, we conclude that β_2-m immunostaining was detected more often in the JP group than in the controls or in patients with chronic periodontitis. This intensified reactivity might be associated with increased production of β_2-m by the epithelial cells or represent the proliferative stage of B cells, or both.

References

1. Berggård I. β_2-microglobulins: isolation, properties and distribution. Fed Proc 1976;35:1167-70.
2. Peterson PA, Cunningham BA, Berggård GM, Edelman S. β_2-macroglobulin, a free immunoglobulin domain. Proc Natl Acad Sci USA 1972;69:1697.
3. Peterson PA, Rask L, Lindholm JB. Highly purified papain solubilized HLA-antigens contain β_2-macroglobulin. Proc Natl Acad Sci USA 1974;71:35–9.
4. Festenstein H, Demant P. HLA and H-2 basic immunogenetics, biology and clinical relevance. Edward Arnold Ltd, London, 1978;10:109–10.
5. Coverna M, Biguzzi S. β_2-microglobulin in normal human tissues. Eur J Immunol 1976;6:830–1.
6. Berggård I, Bearn AG. Isolation and properties of a low molecular weight β_2-microglobulin occurring in human biological fluids. J Biol Chem 1968;243:4096–103.
7. Talal N, Grey HM, Zwaifler N, Michalski JP, Daniels TE. Elevated salivary and synovial fluid β_2-macroglobulin in Sjögren's sndrome and rheumatoid arthritis. Science 1975;187:1196–8.
8. Syrjänen S. Salivary glands in rheumatoid arthritis [Thesis]. Proc Finn Dent Soc 1982;78(suppl III):1–77.
9. Pesce AJ, Weiss MA, Di Persio L, Michael JG. β_2-macroglobulin in cancer. IMJ 1981;160:191–5.
10. Evrin PE, Wibell LL. Serum β_2-macroglobulin in various disorders. Clin Chim Acta 1973;43:183–6.
11. Wibell I, Ervin PE, Berggård I. Serum β_2-microglobulin in renal disease. Nephron 1973;10:320–4.
12. Scully C. Serum β_2 microglobulin in oral malignancy and premalignancy. J Oral Pathol 1981;10:354–7.
13. Grey HM, Kubo RT, Starzl TE. Serum β_2-microglobulin in homografted patients. J Clin Invest 1976;53:30A.
14. Kallioinen M, Dammert K. β_2-macroglobulin in benign and malignant adnexal skin tumors and metastasizing basocellular carcinomas. J Cutan Pathol 1984;11:27–34.
15. Forsum V, Malmnäs Tjernlund U. Epidermal location of beta-2-microglobulin in human skin. Acta Derm Venereol 1977;57:121–3.
16. Mälmnäs Tjernlund U, Forsum U. Beta-2-microglobulin in basal cell carcinoma. Acta Derm Venereol 1977;57:503–6.
17. Lindhe J. Textbook of periodontology. Copenhagen: Munksgaard, 1983.
18. Lever WF, Schaumburg-Lever G. Histopathology of the skin. 5th ed. Philadelphia: J.B. Lippincott Co., 1975;498–561.
19. De Persio LP, Weiss MA, Michael JG, Pesce AJ. Evaluation of the peroxidase and antiperoxidase

method for demonstrating cell membrane β_2-microglobulin. Am J Clin Pathol 1981;77:700–4.
20. Weiss MA, Michael JG, Pesce AJ, Di Persio L. Heterogeneity of β_2-m in human breast carcinoma. Lab Invest 1981;45:46–57.
21. Syrjänen S, Markkanen H, Syrjänen K. Cytokeratins in gingival epithelium of clinically healthy and periodontally diseased subjects. Proc Finn Dent Soc 1984;80:115–20.
22. Nakav T, Matsumoto H, Miyazaki T, Watanabe S, Masaoka T, Takatsuki K, Kishihara M, Kobayashi N, Hattori M, Fujita T. Genetic and clinical studies of serum β_2-microglobulin levels in haematological malignancies. Clin Exp Immunol 1981;46:134–41.
23. Curry R, Thoen J, Shelborne C, Gaudernack G, Messner R. Antibodies to and elevation of β_2-macroglobulin in the serum of ankylosing spondylitis patients. Arthritis Rheum 1982;25:375–80.
24. Child JA, Spati B, Illingworth S, Barnard D, Corbett S, Simmons AV, Stone J, Worthy SR, Cooper EH. Serum β_2-microglobulin and C-reactive protein in the monitoring of lymphomas. Findings in a multicenter study and experience in selected patients. Cancer 1980;45:318–24.
25. Syrjänen S, Markkanen H, Syrjänen K. Inflammatory cells and their subsets in lesions of juvenile periodontitis. A family study. Acta Odontol Scand 1984;42:285–92.

Received for publication 17 September 1984